PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: David Aaron Katz *et al.*

Serial No.: 09/747,538

Filed: December 21, 2000

For: AMPLIFICATION BASED POLYMORPHISM DETECTION

Attorney Docket No.: 6652.US.01

Examiner: Suryaprabha Chunduru

Group Art Unit: 1637

Certificate of Mailing:
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being sent by first class mail to the Commissioner for Patents, PO Box 1450, Alexandria, VA 22313-1450 on January 5, 2006.

Laura Czech

DECLARATION OF MARIA C. GENTILE UNDER 37 C.F.R. SECTION 1.132

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

I, Maria C. Gentile, hereby declare:

1. In December 1993, I received my B.S. in Biological Sciences with a Molecular Biology concentration from the University of Wisconsin-Parkside, Kenosha, Wisconsin. In May 1994, I received my Master's degree in Applied Molecular Biology, also from the University of Wisconsin-Parkside. Since July 1994, I have been employed by Abbott Laboratories as a Research Scientist or Cellular/Molecular Biologist. Currently, I am conducting research in the area of cancer biomarkers and therapeutic antibodies.

2. I am one of the inventors of the above-identified patent application (the "Application"). I was originally listed on the patent with my married name, Maria C. Gentile-Davey. My current legal name is Maria C. Gentile.

3. In the Office Action mailed on August 5, 2005, the Examiner rejected claims 17 and 18 under 35 U.S.C. Section 102(e) as being anticipated by Wittwer et al. (U.S. Patent No. 6,232,079). Claims 38-40 and 43 were rejected under 35 U.S.C. Section 102(b) as being anticipated by Meyer et al. (U.S. Patent No. 5,648,482). Claim 41 was rejected under 35 U.S.C. Section 103(a) as being unpatentable over Meyer et al. in view of Wittwer et al.

4. I conducted an experiment to detect the presence of a mutation known as CYP2D6 star five (hereinafter "*5") in human blood samples obtained from the Interstate Blood Bank, Inc. (Chicago, Illinois) (the samples were designated FAM-A1-FAM-A9). The *5 mutation comprises a deletion of most of the CYP2D6 gene. More specifically, this experiment was conducted to detect

the *5 mutation in samples heterozygous for the *5 mutation or homozygous for the non-variant sequence.

The following primers and probes as identified in the Application were used for the detection of the *5 mutation:

a. SEQ ID NO: 19 – a forward primer specific for CYP2D6;
b. SEQ ID NO: 20 – a forward primer specific for CYP2D7P (a pseudogene of CYP2D6);
c. SEQ ID NO: 21 – a reverse primer that is common for both the CYP2D6 and CYP2D7P sequences;
d. SEQ ID NO: 22 – a molecular beacon probe labeled at its 5' end with fluorescein and dabcyl at its 3' end. SEQ ID NO:22 is perfectly complementary to the CYP2D6 amplification product and has a single base pair mismatch with the CYP2D7P product; and
e. SEQ ID NO: 23 – an unlabeled molecular beacon probe that is perfectly complementary to the CYP2D7P amplification product. SEQ ID NO: 23 was used for the purpose of providing a competitive probe for the CYP2D7P amplification product.

Each of the above primers and molecular beacon probes were synthesized using standard cyanoethyl phosphoramidite chemistry as described in U.S. Patent No. 5,464,746.

The amplification reaction and detection of the amplification product were run in a unit dose format and read in real-time, after each amplification cycle, using a Perkin-Elmer 7700 thermocycler. Reagents for amplification and detection were placed in a single reaction vessel for cycling and detection. Specifically each 50 µl reaction contained 1x Gibco BRL PCR buffer (Gibco, Inc.; Grand Island, NY), 1.5 mM magnesium chloride, 0.2 mM dNTPs, 2.5 units of Gibco BRL Platinum Taq polymerase, 0.1 µM of each primer, 0.1 µM of each probe, 12.5 ng of genomic sample DNA and 0.15 µl of Texas-Red conjugated heptanucleotide control.

Individual reaction vessels were placed in the thermocycler and 45 cycles of the following cycle were performed: 60 seconds at 94°C, 20 seconds at 59°C, 40 seconds at 61°C, and 40 seconds at 72°C. A fluorescent reading was taken at the 61°C step of each cycle.

Sample designations along with the cycle number at which a fluorescent reading was detectable over a given threshold value (the dark black line) is shown in the amplification plot attached as Exhibit A. Samples that do not contain the *5 mutation are shown in purple and samples that contain the *5 mutation are shown in orange. The control is shown in red. Exhibit A shows that samples containing the *5 mutation (the orange signal) were detected in later cycles than those that did not contain *5 mutation (the purple signal). As

explained above, the reason for this is that the samples that did not contain *5 contained a greater proportion of target sequence for amplification and thus were detected earlier. Also, the amplification plot in Exhibit A clearly shows the ability of the method to resolve samples that did not contain the *5 mutation from those samples that do contain the *5 mutation.

5. The detection of the presence of the*5 mutation in human blood samples designated FAM-A1-FAM-A9 was repeated by me as described above in Paragraph 4 with one modification in the PCR cycle. Specifically, after the individual reaction vessels were placed in the thermocycler, 45 cycles of the following cycle were performed: 60 seconds at 94°C, 60 seconds at 59°C and 40 seconds at 72°C. In contrast to the PCR cycle described in Paragraph 4, this cycle did not include a step of 40 seconds at 61°C. A fluorescent reading was taken at the 59°C step of each cycle.

The amplification plot for sample designations FAM-A1 though FAM-A9 are shown in attached Exhibit B. Samples that do not contain the *5 mutation are shown in purple and samples that contain the *5 mutation are shown in orange. The control is shown in red. In contrast to the amplification plot shown in Exhibit A, not all the samples containing the *5 mutation were detected in later cycles than those samples that did not contain the *5 mutation. Also, the resolution of the method in the amplification plot shown in Exhibit B between samples that are heterozygous for the *5 mutation from those samples that did not contain the *5 mutation is not clear, particularly when compared to the amplification plot shown in Exhibit A.

6. The detection of the presence of the *5 mutation in human blood samples designated FAM-A1-FAM-A9 was repeated by me as described above in Paragraph 4 with a second, different modification in the PCR cycle than that described in Paragraph 5. Specifically, after the individual reaction vessels were placed in the thermocycler, 45 cycles of the following cycle were performed: 60 seconds at 94°C, 60 seconds at 61°C and 40 seconds at 72°C. In contrast to the PCR cycle described in Paragraph 4, this cycle did not include a step of 40 seconds at 59°C. A fluorescent reading was taken at the 61°C step of each cycle.

The amplification plot for sample designations FAM-A1 though FAM-A9 are shown in attached Exhibit C. Samples that do not contain the *5 mutation are shown in purple and samples that contain the *5 mutation are shown in orange. The control is shown in red. In contrast to the amplification plot shown in Exhibit A, not all the samples containing *5 mutation were detected in later cycles than those samples that did not contain the *5 mutation. Also, the resolution of the method in the amplification plot shown in Exhibit C between samples that are heterozygous for the *5 mutation from those samples that did not contain the *5 mutation is not clear, particularly when compared to the amplification plot shown in Exhibit A.

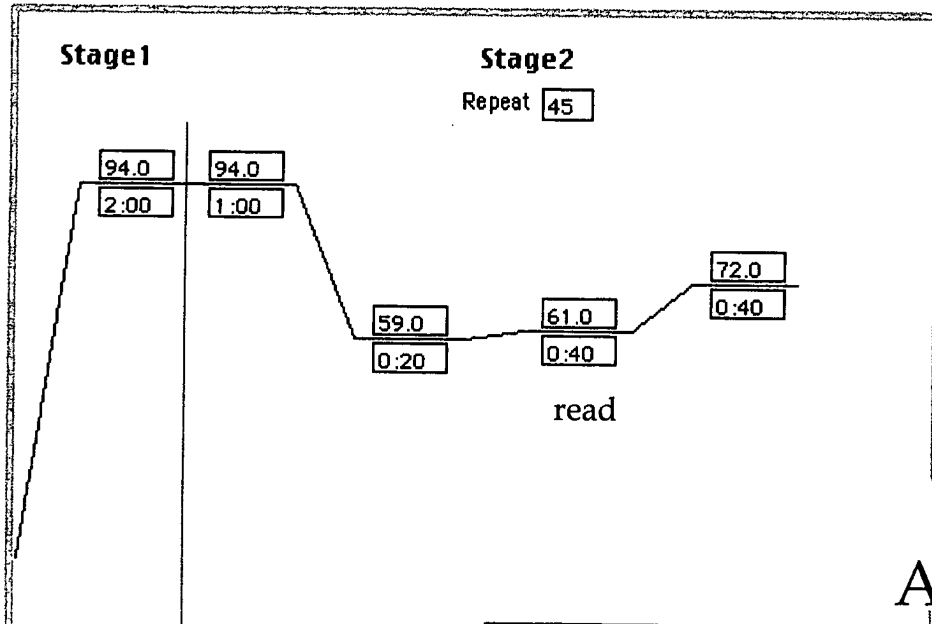
7. I hereby declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Maria C. Gentile

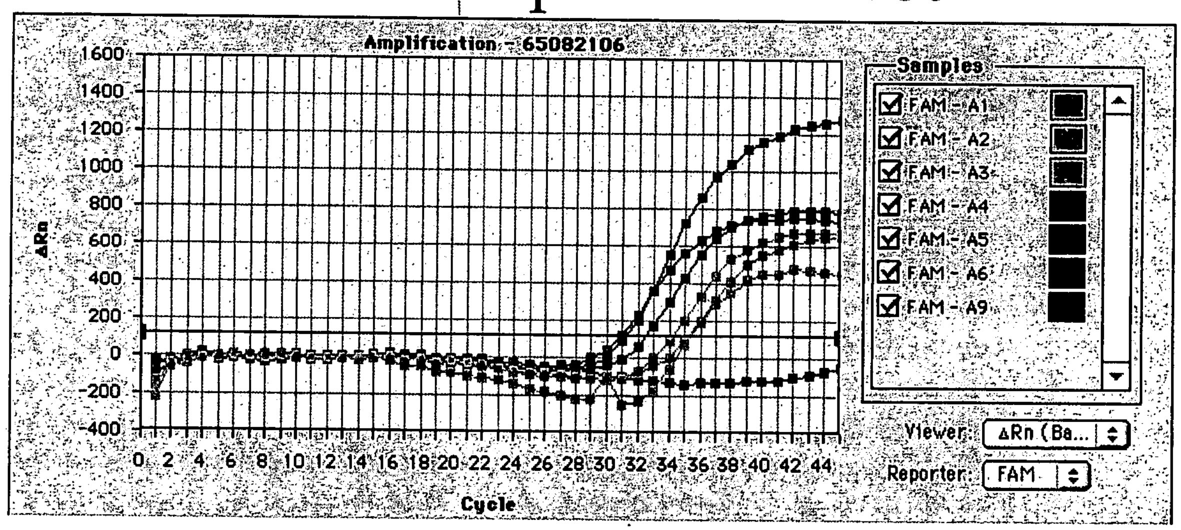
1-5-06
Date

EXHIBIT A

Thermal Cycling Profile



ABI-Prism 7700 Amplification Plot



CYP2D6 - two copies/genome

CYP2D6 - one copy/genome

Water

Thermal Cycling Profile

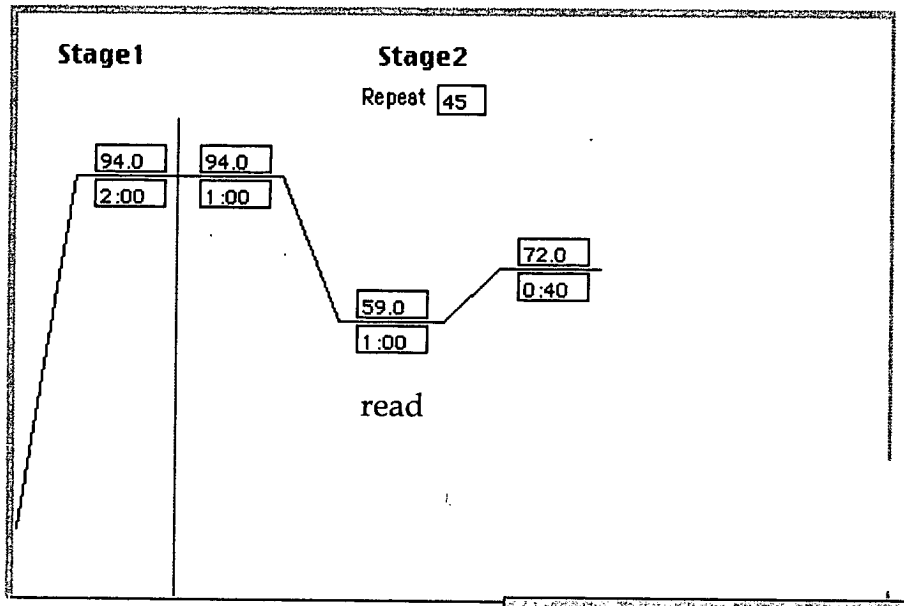


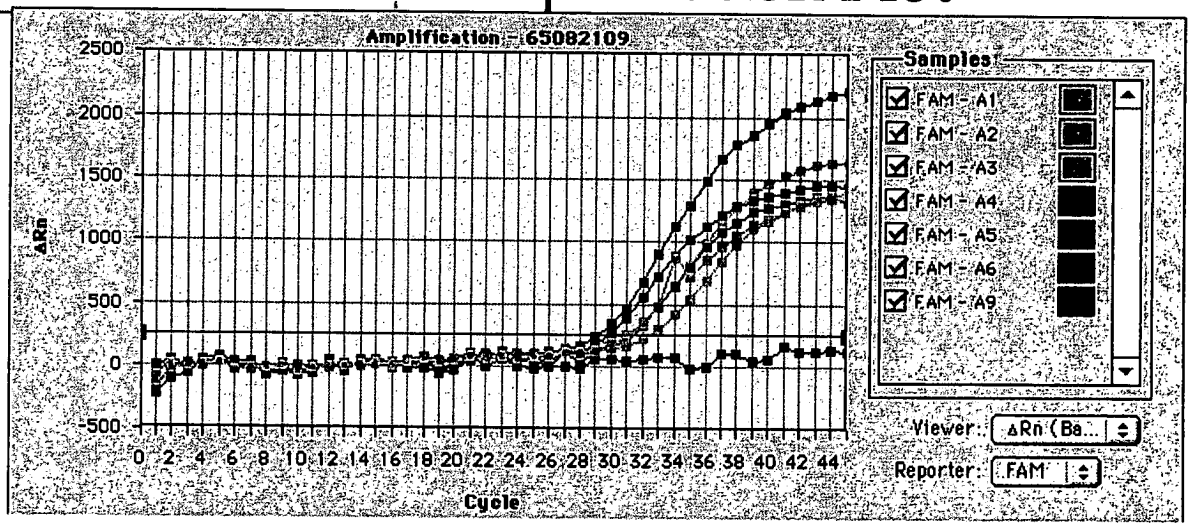
EXHIBIT B

ABI-Prism 7700 Amplification Plot

CYP2D6 - two copies/genome

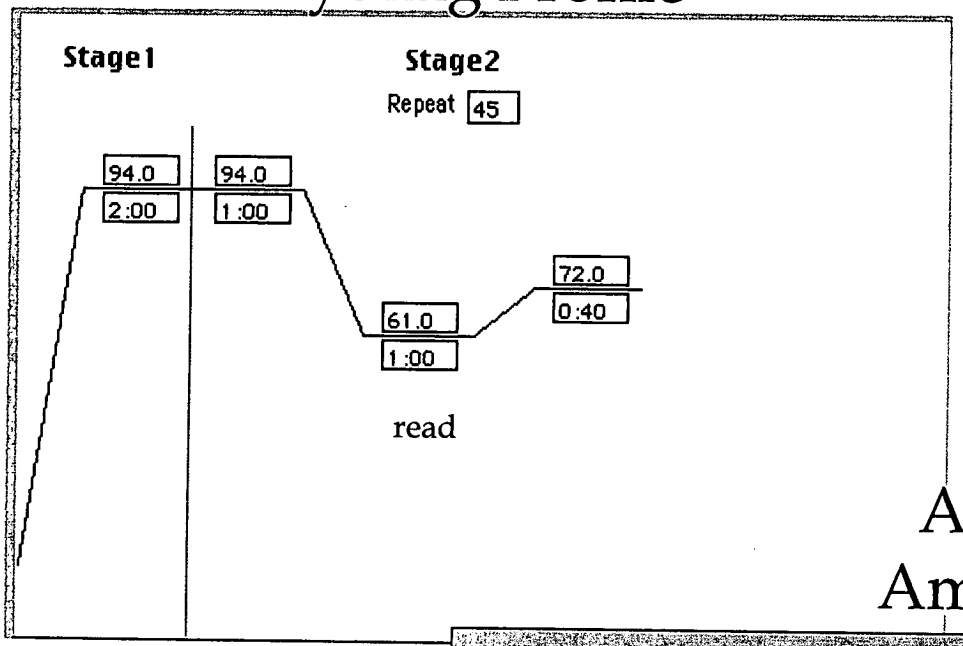
CYP2D6 - one copy/genome

Water



Thermal Cycling Profile

EXHIBIT C



ABI-Prism 7700 Amplification Plot

CYP2D6 - two copies/genome

CYP2D6 - one copy/genome

Water